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BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 81, NO. 6, PP. 489–491 NOVEMBER, 1954

THE CYTOLOGY OF XANTHISMA TEXANUM D. C. I. DIFFERENCES IN THE CHROMOSOME NUMBER OF ROOT AND SHOOT.[1]

C. A. BERGER AND E. R. WITKUS

The chromosome number of *Xanthisma texanum,* Star of Texas, a composite was determined from root tip smears to be $2n = 8$, (figs. 1 and 2). While attempting to confirm this determination by studying meiotic divisions in pollen mother cells, it was found that some plants have four bivalents at the 1st meiotic division (figs. 5–9) while others have five (figs. 10–13). The situation was further complicated when it was found that the root tip cells of five-bivalent plants do not have 10 chromosomes but have 8 as do the four-bivalent plants. The plants on which these observations were made were raised from seeds obtained from the New York Botanical Garden. Seeds were obtained from three other sources (Burpee, Pearce, and Park Seed Companies). Plants raised from these varied sources all showed the same cytological conditions; some having four bivalents while others had five, yet all with eight chromosomes in the root tip cells.

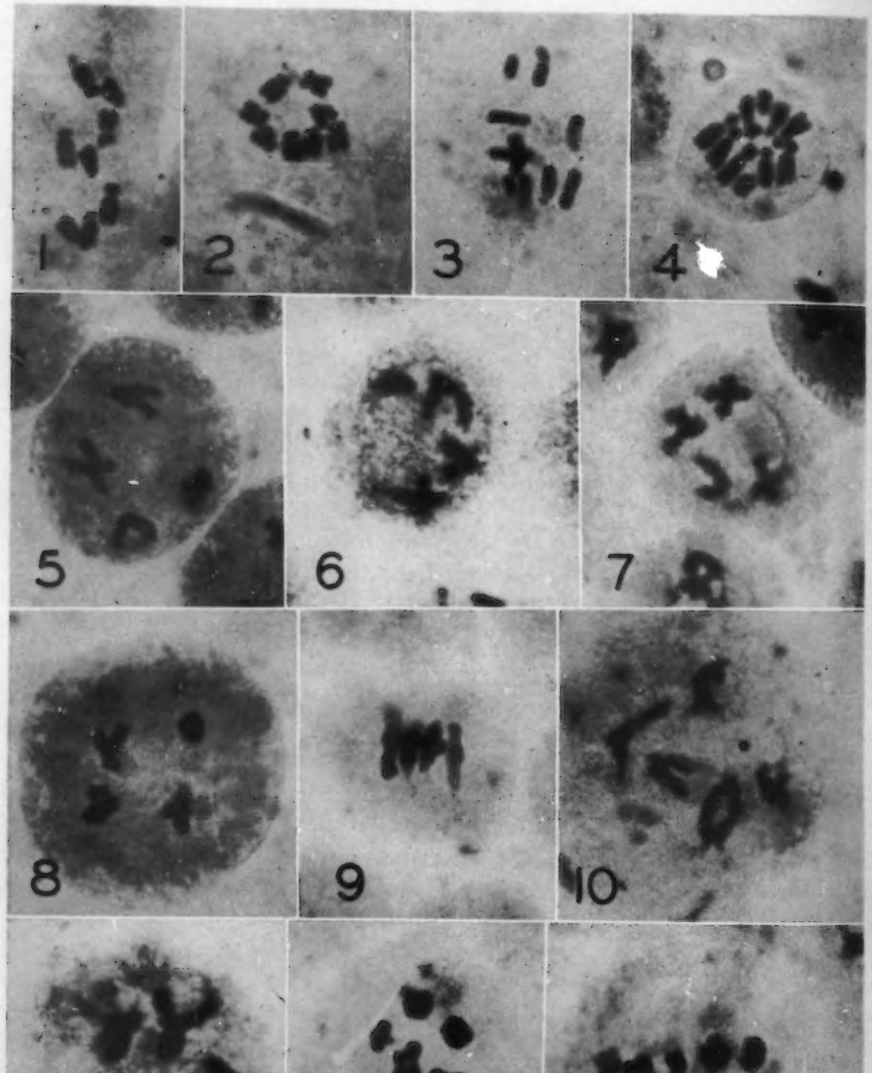
It was noted that the tapetal cells of five-bivalent plants had 10 chromosomes and that dividing cells of the non-germinal anther tissue of these plants also had 10 chromosomes (fig. 4). These findings suggested that the differentiation was not between germ cells and somatic cells but between shoot and root. Accordingly many young seedlings were germinated and were found to fall into two classes. In one group the cells of both root and shoot had 8 chromosomes while in the other group the cells of the root had 8 chromosomes but all dividing cells of the shoot showed 10 (fig. 3).

Apparently, therefore, populations of *Xanthisma texanum* are composed of two cytologically different types, one having the chromosome number of 8 in the cells of the entire plant, the other having 8 chromosomes in the cells of the root and 10 chromosomes in the cells of the shoot, including the germ cells. The two types of plants are morphologically indistinguishable.

The four chromosomes of the haploid complement are readily distinguishable. The two smaller chromosomes are 3.1 μ in length, both are J-shaped but one has a much smaller short arm than the other. The two larger chromosomes are 3.8 μ in length, they too are J-shaped and are easily distinguished by the length of the short arm. The karyotype is shown in

[1] This investigation was begun with the aid of a research grant (PHS C-492 (C5)) from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

[THE BULLETIN for September–October, 1954 (81: 387–487) was issued October 25, 1954.]



FIGS. 1–13. FIGS. 1 and 2. Metaphase in root tip cells, $2n = 8$. FIG. 3. Somatic metaphase from shoot of seedling, $2n = 10$. FIG. 4. Metaphase of somatic cell in another wall, $2n = 10$. FIGS. 5–8. Diakinesis in microsporocytes of 4-bivalent plants. FIG. 9. 1st metaphase in microsporocyte of 4-bivalent plant. FIGS. 10–12. Diakinesis in microsporocytes of 5-bivalent plants. FIG. 13. 1st metaphase in microsporocyte of 5-bivalent plant. (All figures × 1500.)

figure 14. Of the two smaller chromosomes, the one with the shortest small arm has been designated as Chromosome I, the other as Chromosome II. Likewise, of the two larger chromosomes, the one with the shortest small arm has been designated as Chromosome III, the other as Chromosome IV.

When somatic divisions in the shoot of five-bivalent plants were studied, it was found that the 'extra' chromosome (Chromosome V) did not correspond to any of the four chromosomes of the haploid complement. It is smaller than any of the four, apparently consists of a single arm and is the same size as the long arm of Cromosome II.

Preliminary studies of adventitious roots from shoot cuttings of four- and five-bivalent plants showed that the chromosome number of the cells of adventitious roots corresponds with the chromosome number of the shoots from which they arose.

Discussion. Many cases of differences in the chromosome number within a species have been reported in plants. Some of these cases have been shown,

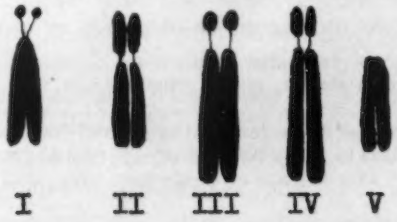


FIG. 14. Idiogram of *Xanthisma texanum*. Chromosome V is not present in 4-bivalent plants.

by their meiotic pairing behavior, to be due to fragmentation or fusion, as when a V-shaped chromosome pairs with two rod-shaped chromosomes. We have found no evidence that the extra chromosomes of *Xanthisma texanum* are of this type. Chromosome V has never been observed to pair with any of the other four.

Other reported cases have involved extra chromosomes called supernumerary, accessory or B-type chromosomes by various authors. *Xanthisma* appears to resemble the B-type chromosome of corn, with one very important difference. Apparently none of the previously reported cases involved a difference in chromosome number between the cells of the root and those of the shoot of the same plant.

The early embryology of *Xanthisma* is now being studied to ascertain how and when this difference in chromosome number arises.

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BULLETIN OF THE TORREY BOTANICAL CLUB
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SPECIES OF CORDYCEPS ON SPIDERS[1]

E. B. MAINS

In 1953 three very interesting specimens of a *Cordyceps* on spiders were received from Dr. Rolf Singer who had collected them in Tucuman, Argentina. They proved to be a new species. In the course of the study a specimen of another undescribed species on a spider from Grenada was discovered in the Farlow Herbarium. The results of the study of available collections of *Cordyceps* on spiders and of a survey of the literature have been included in this paper. The herbaria in which the specimens are deposited are designated as follows: (MICH) Herbarium of the University of Michigan; (FH) Farlow Herbarium, Harvard University.

KEY TO THE SPECIES OF CORDYCEPS ON SPIDERS

1. Perithecia superficial, not imbedded in a stroma 2
1. Perithecia imbedded in a stroma, either completely or with upper portions protruding 3
2. Ascocarps light brown; perithecia few, scattered; asci 600–780 μ long ..... *C. thaxteri*
2. Ascocarps brownish black or black below, flavo-cinereus above; perithecia crowded in groups near the apices of the ascocarps; asci 300–400 μ long ..... *C. engleriana*
3. Ascocarps narrowly cylindric or linear, 3 cm. or more long; prithecia imbedded except for the ostiole 4
3 Ascocarps clavate or subcapitate, less than 3 cm. long; perithecia protruding from a soft stroma 7
4. Ascocarps bearing a number of conidial branches at the base ..... *C. arachneicola*
4. Ascocarps not bearing conidial branches 5
5. Perithecia flask-shaped with narrow necks, 850–1200 × 220–270 μ ..... *C. cylindrica*
5. Perithecia ovoid or ellipsoid, smaller, 250 × 150 μ or less 6
6. Ascocarps purplish brown, up to 6 cm. long; perithecia 100–140 × 60–75 μ; asci 70–100 × 4 μ ..... *C. ignota*
6. Ascocarps brownish red to cinnabar, 9–11.5 cm. long; perithecia 215–250 × 100–150 μ; asci 90–135 × 3–4 μ ..... *C. caloceroides*
7. Perithecia 336–360 × 156–216 μ; asci 180–252 × 4 μ; ascospores 180–252 × 1 μ, not breaking into part spores ..... *C. grenadensis*
7. Perithecia 325–550 × 200–500 μ; asci 300–450 × 3.5–4 μ; ascospores breaking into part spores, 3–4 × 0.7–1.0 μ ..... *C. singeri*

Cordyceps thaxteri Mains Jour. Elisha Mitchell Soc. **55**: 120. 1939. (Figs. 1 & 2). Ascocarps subcylindric to narrowly clavate, somewhat narrowing below, small; 1.5–2.5 mm. long, 0.1–0.2 mm. thick below, up to 0.3 mm. thick above, light brown; perithecia few, 1–4, scattered, superficial, free, narrowly ovoid, large, 960–1200 × 300–360 μ, more or less tomentose over the lower portion; asci narrowly cylindric, 600-780 × 6–7 μ, wall thin, thickened at the apices; ascospores filiform nearly as long as the asci, 1–1.5 μ

[1] Illustrations published with the assistance of the Lucien M. Underwood Memorial Fund.

thick, mutiseptate, with cells 6–10 μ long, tardily breaking into one-celled segments.

Conidial stage: *Akanthomyces aranearum* (Petch) Mains. Mycologia **42**: 574. 1950. *Hymenostilbe aranearum* Petch Trans. British Myc. Soc. **16**: 221. 1932.

Synnemata simple or occasionally sparsely branched, cylindric to narrowly clavate, 0.8–10 mm. long, 0.1–0.2 mm. thick, brown, sometimes pubescent below due to short asperulate hyphae white pulverulent above from adhering conidia; phialides arising as lateral cells from the outer hyphae of the synnemata, obovoid or ellipsoid, 6–12 × 4–8 μ, rounded above and abruptly narrowing into a short sterigma, asperulate; conidia narrowly obclavate, 8–14 × 1.5–3 μ, often acute at the lower end, narrowing upward, rounded or obtuse at the upper end, one-celled, hyaline, smooth, catenulate.

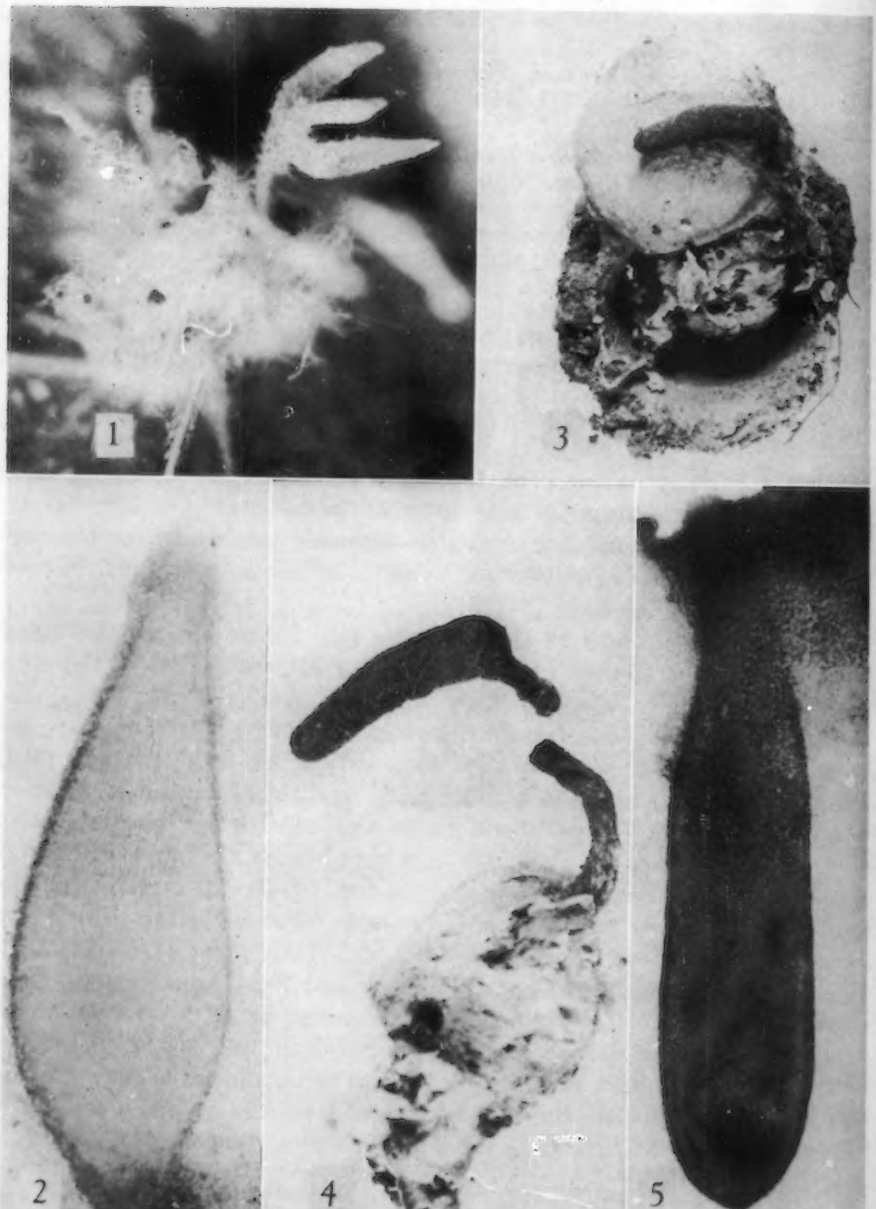
TYPE: On spiders Cranberry, N .C., R. Thaxter (FH 4123), isotype (MICH).

Specimens studied: Maine (FH 649, 6258); North Carolina (FH 665, 4123,); Tennessee (FH 4122A).

This species is unique. The few large perithecia are borne on small ascocarps, the perithecia in some instances being nearly as large as the ascocarp (fig. 1). Synnemata are more commonly produced than the ascocarps. The catenulate conidia accumulate in white pulverulent masses (Mains 1950b, figs. 5–6, 19 A–C).

Cordyceps engleriana P. Henn. Engler Bot. Jahrb. **23**: 538. 1897 with figs. 3 a–g.[1] This species was described and illustrated by P. Hennings from a specimen on a spider from Kamerun, Africa. Petch (1934) has also reported the species from British Guiana. These specimens have not been studied. From the published information the ascocarps are many (Hennings fig. 3a) up to 15 mm. long, .25 mm. thick, flavo-cinereus above and blackish below. The perithecia are superficial, free but crowded on one side of the ascocarp or clustered in a group near the apex (Hennings fig. 3b). They are ovoid or flask-shaped, 600 × 300 μ. The asci are cylindric 300–400 μ long and 8–10 μ (Hennings) or 5 μ (Petch) wide. The ascospores are filiform breaking into 22–25 × 1.5–2 μ cylindric fragments (Hennings figs. 3d and e). Both Hennings and Petch state that the ascocarps are accompanied by synnemata (Hennings figs. 3 f. and g.), which are black, simple or branched, up to 15 mm. long, and 200 μ thick below and with branches dark brown, 45 μ thick. The phialides are lateral, cylindric 6–7 × 3.5–4 μ (Hennings), 18 × 3 μ (Petch). The conidia are ovoid-ellipsoid, 4–5 × 3–3.5 μ and hyaline. Petch states that the conidial stage is a *Hymenostilbe*.

[1] Rogers (1954) has pointed out that when Link established *Cordyceps* on a generic basis he treated it as a masculine noun and that according to Recommendation 83A(3) of the International Rules of Nomenclature it should be so considered. However, it has otherwise generally been treated as feminine and it is doubtful therefore whether it is desirable to make the change since it is not a requirement.



FIGS. 1–2, *Cordyceps thaxteri* type. FIG. 1, small spider bearing an ascocarp with two suprficial perithecia × 10. FIG. 2, perithecium × 75. FIGS. 3–5, *Cordyceps cylindrica* type. FIG. 3, nest of trapdoor spider with lid open, spider covered with mycelium within and upper portion of ascocarp placed on the lid × 1.3. FIG. 4, spider removed from the nest showing mycelial covering and broken ascocarp × 2. FIG. 5, perithecium removed from stroma with portion of stroma adhering to the neck × 75.

Petch has concluded that *C. flavo-viridis* Möller is probably on a spider and is the same as *C. engleriana*. Möller (1901) described the species on brown pupae from Brazil. His illustration shows white strands appressed to the substratum bearing yellowish green superficial perithecia. The species appears to be a *Torrubiella*.

C. engleriana differs from *C. thaxteri* in the color of the ascocarps. The perithecia are more numerous and are crowded together in compact groups usually near the apices of the ascocarp. The asci are much shorter and the cells of the ascospores are much longer.

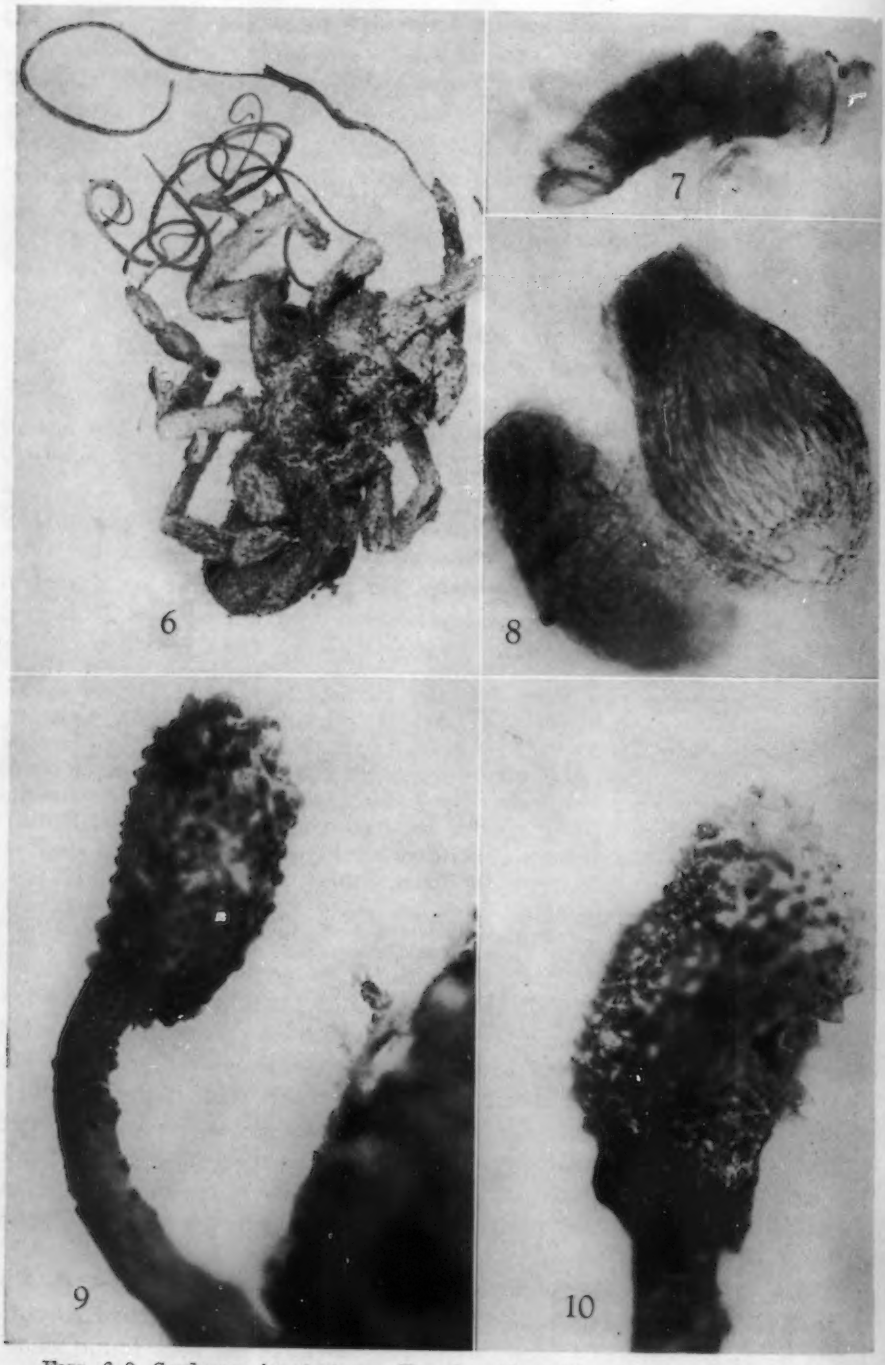
Cordyceps arachneicola Kobayasi Sci. Tokyo Bun. Daig. 5 no. 84: 123–125. 1941. This species was described on a spider from Japan. The ascocarp is given as cylindric up to 5 cm. long and 2 mm. thick and with the ascogenous portion 1 cm. long, tawny-olive, not distinct from the stipe. The perithecia are described as ellipsoid, completely imbedded. It is stated that the asci are immature. In proposing the species Kobayasi places great emphasis on the conidial stage which he states occurs on branches from the lower portion of the ascocarp. The conidial hyphae are described and illustrated as occurring in a layer covered by a peridium. This is a most unusual conidial condition.

Cordyceps cylindrica Petch Trans British Myc. Soc. **21**: 46. 1937. (Figs. 3–5). Ascocarp brownish black, cylindric capitate; stipe approximately 15 mm. long, 1.5–2.0 mm. thick somewhat twisted (white when fresh); head somewhat oblique, abruptly enlarged, subcylindric, 15 mm. long, 3 mm. wide below, 2.5 mm. wide above, rounded at the apex, minutely punctate from the ostioles (pale yellow when fresh); perithecia entirely imbedded at right angles to the surface, narrowly ovoid or flask shaped, 850–1200 × 220–270 μ, gradually narrowing above into a neck 60–80 μ wide; asci and ascospores not seen.

On trapdoor spider, St. Anne's Valley, Trinidad, Aug. 22, 1923, D. H. Linder no. 111, type (FH).

Through the kindness of I. Mackenzie Lamb the type from the Farlow Herbarium has been available for study. The spider occurs in a somewhat irregular nest and is covered with a pinkish mycelium (figs. 3 & 4). The single ascocarp is broken a short distance below the head. It probably projected from between the lid and side of the nest. A note on the specimen indicates that the stipe was white and the head a pale yellow when fresh. The dried specimen is brownish black. *C. cylindrica* is distinguished from the species with imbedded perithecia by its long perithecia with narrow necks (fig. 5).

Petch (1939) has suggested that *Isaria atypicola* Yasuda is the conidia stage. This is based on the similarity of the shape of the synnemata of the *Isaria* to the ascocarp of *C. cylindrica*. The two have not been found associated.



FIGS. 6–8, *Cordyceps ignota* type. FIG. 6, spider with ascocarps arising from legs ×1.3. FIG. 7, portion of stroma showing crowded perithecia ×100. FIG. 8, perithecia ×350. FIGS. 9 and 10. *Cordyceps grenadensis* type. FIG. 9, ascocarp showing head and portion of stipe ×20. FIG. 10, head of the other ascocarp showing protruding perithecia ×30.

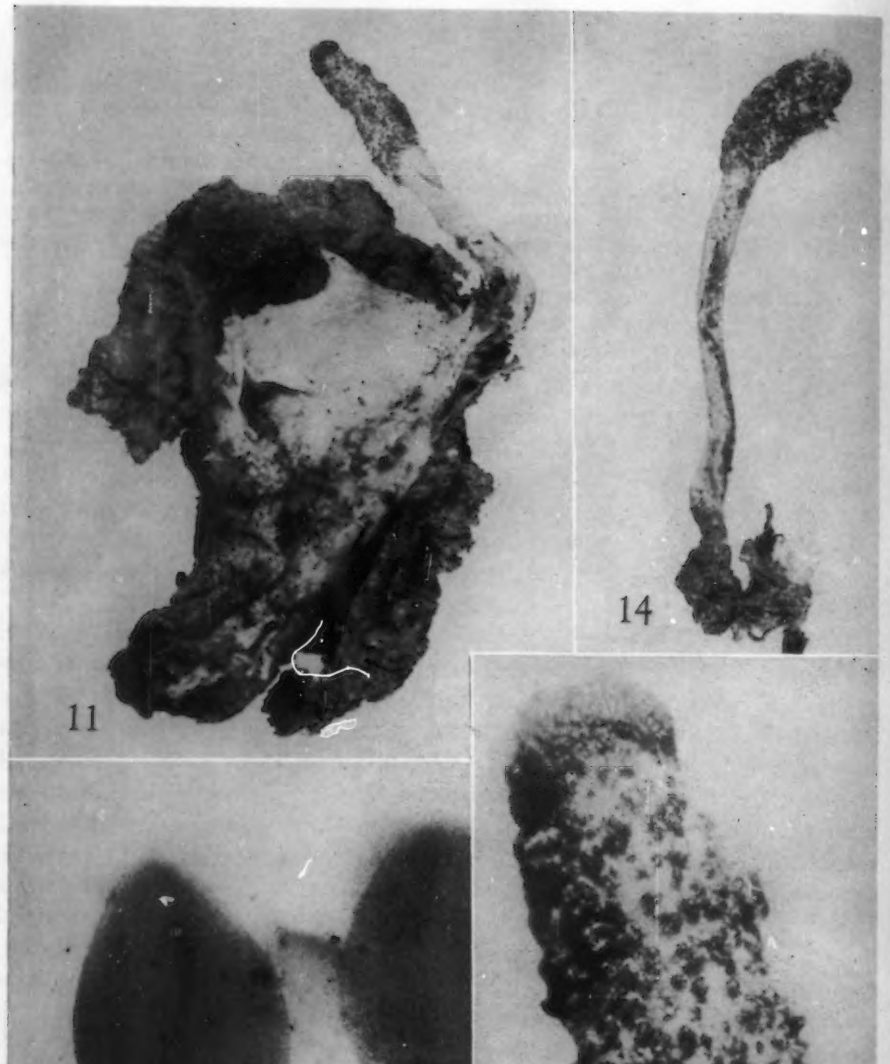
Cordyceps ignota March. Physis **20**: 17. 1954. (Figs. 6–8). Ascocarps simple or branched, slender, up to 6 cm. long. terete or somewhat compressed below, 0.5–1.5 mm. thick, acuminate at the apices, purplish brown, minutely punctate above from the ostioles of the perithecia; perithecia very crowded in a compact layer on the upper third of the ascocarps, imbedded in a slight, loose stroma, ovoid, 100–140 × 60–75 μ; asci narrowly clavate, 70–100 × 4 μ, the wall thin, thickened at the apices; ascospores immature.

On a large spider (Theraphoridae), Argentina, A. Ibarra Grasso, Oct. 16, 1944, type, in the Herbarium of the Division of Fitopathologia, Ministerio de Agricultura, Buenos Aires, Argentina.

Through the kindness of Jorge Wright the type of this species was loaned for this study. The ascocarps are very long and slender and arise mostly from the legs of the host (fig. 6). Mature ascospores were not seen. They are described by Marchionatto as filiform, 59–61 × 2 μ and continuous. It is probable that when mature the ascospores are multiseptate and do not break into fragments. The perithecia are very small and are very closely crowded together with only a small amount of a loose stroma between them (figs. 7 and 8).

Cordyceps caloceroides Berk. & Curt. Jour. Linn. Soc. **10**: 375. 1869. *Cordyceps wittii* P. Henn. Engler. Bot. Jahrb. **23**: 539. 1897. *Ophiocordyceps caloceroides* (Berk. & Curt.) Petch. Brit. Myc. Soc. Trans. **18**: 63–64. 1933. Berkeley and Curtis based their description on a specimen collected by C. Wright (309) in Cuba. It was collected without the host. Petch (1933, 1937) has decided that it probably was on a spider since he has concluded that it is the same as *C. wittii* which was described on a spider. Berkeley and Curtis describe the ascocarps as bright red, furcate, nearly 5 in. long and less than a line thick and the perithecia as immersed with prominent ostioles. R. W. G. Dennis has kindly examined the type in the Herbarium of the Royal Botanic Gardens, Kew. According to notes received from him, it consists of a furcate ascocarp with two branches arising from a whitish mycelial strand on which there is a stub indicating that it probably bore another ascocarp. The overall length is 11.5 cm. and the branches are 6 and 8 cm. long. The stipe is 1 mm. thick and the ascogenous portion up to 2 mm. narrowing to acuminate apices. Dennis states that the specimen is now dark reddish brown but probably had been treated with an alcoholic solution of mercuric chloride which has affected the color. The perithecia form a continuous layer on the upper portion of the branches and there is no sharp separation from the lower sterile portion. From a small fragment received from Dr. Dennis it was found that the perithecia are immersed except for the ostioles, ovoid, 215–250 × 100–150 μ and the asci cylindric, 90–135 × 3–4 μ. No clearly differentiated ascospores or part-spores were found.

P. Hennings described and illustrated *C. wittii* (Hennings pl. 14, figs. 4a–c) from a specimen collected by E. Witt in Ecuador The four ascocarps



FIGS. 11–13, *Cordyceps singeri* type. FIG. 11, nest of trapdoor spider cut open showing mass of mycelium covering spider and the ascocarp ×4. FIG. 12, portion of the head showing protruding perithecia ×20. FIG. 13, perithecium ×75. FIG. 14, *Cordyceps singeri*, one of the ascocarps of collection T 2002, ×4.

are given as cinnabar-red, simple or furcate up to 9 cm. long and 2 mm. thick (published as 2 cm., an obvious error). There is no sharp separation of the ascogenous portion from the lower sterile portion. The perithecia are described as semi-immersed, hemispherical, the asci cylindric-oblong 50 × 14 μ, and the ascospores filiform or subfusoid, nonseptate, 40–45 × 1 μ, not fragmenting. Petch (1933) who examined the type in the Berlin Herbarium states that the perithecia are immersed with slightly protruding ostioles. He gives the perithecia as ovoid, 200 × 60–80 μ, the asci as cylindric 80 × 4 μ and the ascospores as linear, slightly attenuated to each end. 40–45 × 1.5 μ, multiseptate and not dividing into part-spores. Petch's conclusion that *C. wittii* and *C. caloceroides* are the same species appears to be justified. He transfers the species to Ophiocordyceps on account of the non-fragmentation of the ascospores. The generic separation does not appear justified.

Petch (1937) has reported *C. caloceroides* from Grenada. B.W.I. (FH 6122) and Liberia (FH Linder 1093). The latter has clavate ascocarps only 15 mm. long which are immature without perithecia. It cannot be determined. The specimen from Grenada is a new species as follows.

Cordyceps grenadensis sp. nov. (Figs. 9 and 10). Ascomata rubra, subcapitata, 10–12 mm. longa, 1.5–2 mm. crassa, stipitibus gracilibus, 0.5 mm. crassis; perithecia rubra, ovoidea, 336–360 × 156–216 μ semiimmersa; asci clavati, 180–252 × 4 μ, ascosporis cylindricis, 50–70 × 1 μ, multiseptatis, nonfrangentibus.

In aranea, Grand Etang, Grenada, R. Thaxter, Farlow Herb. 6122, specimen typicum.

Ascocarps 2, red, subcapitate, 10–12 mm. long, the ascogenous portion ovoid to cylindric, 1.5–2 × 1 mm. sharply separated from the stipe, the stipes slender 0.5 mm. thick; perithecia red, ovoid, 336–360 × 156–216 μ, partly imbedded in a soft yellowish-red stroma, projecting ⅓ to ½; asci narrowly clavate, 180–252 × 4 μ; ascospores cylindric, 50–70 × 1 μ, multiseptate, the cells 4–5 μ long, not breaking into part-spores.

On ground spider, Grand Etang. Grenada. B.W.I., 1912–1913, A. Thaxter, FH 6122, type.

This specimen has been reported by Petch (1937) as *C. caloceroides*. However as a comparison with that species will show *C. grenadensis* is a very much smaller species. Size of ascocarps in species of *Cordyceps* depends to some extent on the size of the host. However the differences between *C. caloceroides* and *C. grenadensis* are much greater than would be expected for this reason. Also the ascogenous portions of the ascocarps of *C. genadensis* are sharply delimited from the stipes and the perithecia are imbedded in a soft stroma and project for ⅓ to ½ their lengths in the dried specimen. It is probable that the stroma shrinks considerably in drying and that the perithecia are more completely imbedded in the fresh condition.

Cordyceps singeri sp. nov. (Figs. 11–14). Ascomata clavata vel subcapitata, 3–12 mm. longa, supra 1–3 mm. crassa, pallide brunnea vel rubra, stipitibus 0.2–2.0 mm. crassis, brunneo-griseis vel pallide brunneis; peri-

thecia ovoidea, 325–550 × 200–500 μ, semiimmersa, brunneo-rubra; asci cylindrici, 300–450 × 3–4 μ, ascoporis filiformibus, multiseptatis, in unicellulares fragmentas secedentibus 3–4 × 0.7–1.0 μ.

In araneis, Tucuman, Argentina, R. Singer, T 1714, specimen typicum (MICH).

Ascocarps clavate or subcapitate, 3–12 mm. long, the upper ascogenous portion obovoid to cylindric, 1–3 mm. wide, light brown to red, rough from projecting perithecia, the stipes 0.2–2.0 mm. thick, at first white, then brownish grey or light brown; perithecia ovoid, 325–550 × 200–500 μ, brownish red, embedded in a soft loose stroma with the upper portions more or less projecting; asci cylindric, 300–450 × 3–4 μ, the wall thin, thickened at the apices into hemispherical caps, 1.5–3 μ thick; ascospores filiform, nearly as long as the asci, hyaline, multiseptate, finally breaking into one-celled fragments, 3–4 × 0.7–1.0 μ.

On trapdoor spiders, Tucuman, Argentina, Rolf Singer: Parque de Aconquija, Sierra de San Javier, Nov. 24, 1950, T 1027; Taff, Parque de Aconquija Jan. 9, 1952, T 1714, type; Taff, Sierra de San Javier, May 1, 1952, T 2002 (MICH).

In the type specimen the host was collected in the nest. It is covered with a white mycelium and the ascocarp project above the lid of the nest (fig. 11). In the other specimens only fragments of the hosts and portions of the nest occur.

In size of ascocarps and in the arrangement of perithecia *C. singeri* is similar to *C. grenadensis*. It has larger perithecia and asci and the ascospores at maturity break into part-spores. Due to shrinkage of the stroma on drying the perithecia are probably more completely imbedded in the fresh condition.

Several other species of *Cordyceps* have been described on spiders. *C. gonylepticida* Möller, *C. flavoviridis* Möller and *C. arachnophila* Johnston are species of Torrubiella (Petch 1937, Mains 1950a). *C. montagnei* Berk. & Curt. and *C. gigantea* (Mont.) Mass. are names which have been applied to *Isaria gigantea* Mont. although the ascogenous stage is unknown.

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THE RELATION OF THE CYTOPLASMIC INCLUSIONS TO THE ESTABLISHMENT OF TETRAHEDRAL SYMMETRY IN THE SPORE QUARTET OF OSMUNDA REGALIS

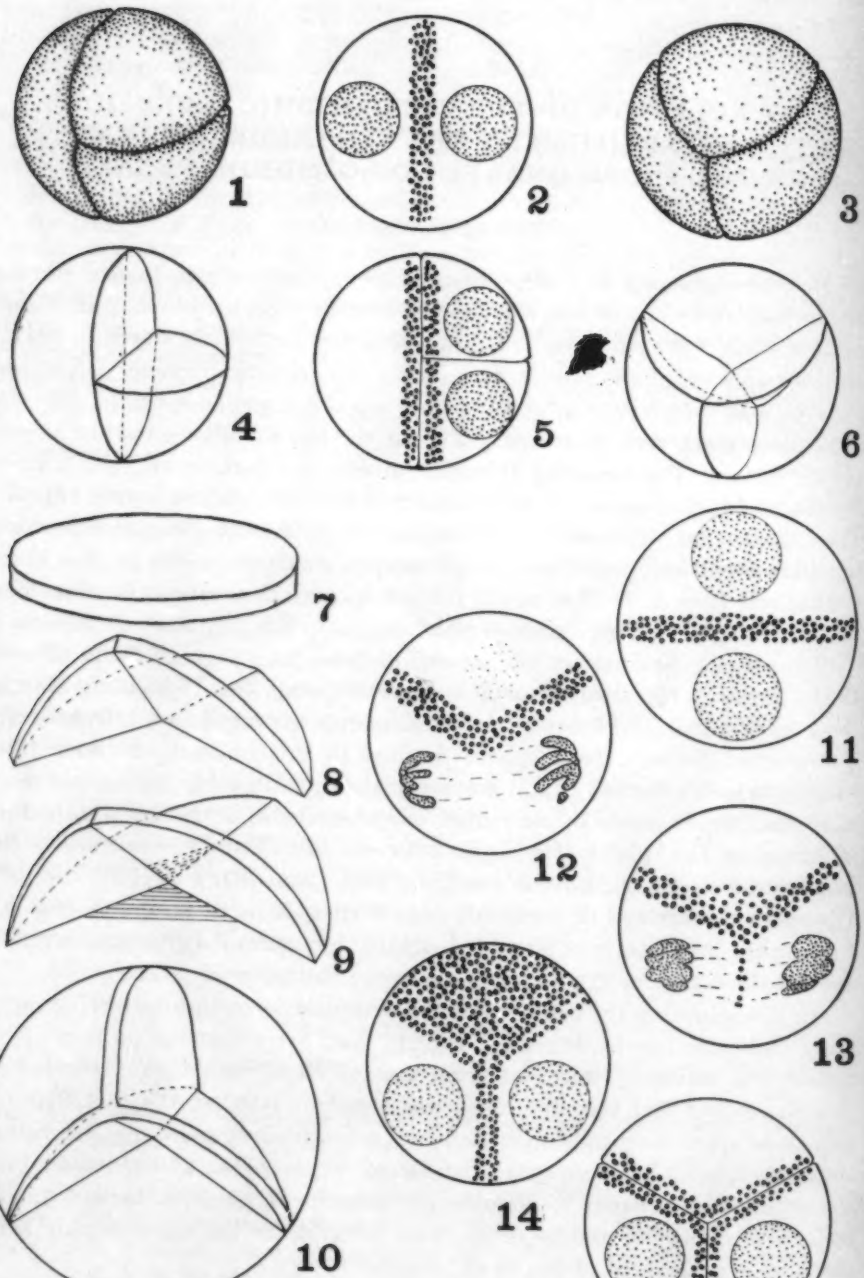
NORMAN P. MARENGO

Precise regularity in the behavior of the cytoplasmic inclusions during the meiotic divisions of sporogenesis in ferns has been described in *Nephrodium molle* (Senjaninova 1927) and *Onoclea sensibilis* (Marengo 1949). In these species, at the termination of the first meiotic division, a compact mass of mitochondria is interposed between the interphase nuclei (fig. 2). This plate maintains its identity during the second division and the cell plates between the resulting telophase nuclei are formed independent of the plate of inclusions which at this stage is divided into two layers (fig. 5). The spindle axes of the second division are at right angles to each other and the four resulting spores are wedge shaped quarter-spheres of the spore mother cell (figs. 1, 4). The newly formed spores are arranged in two pairs, with the long axes of one pair at right angles to the long axes of the other.

The other type of spore mother cell division found in the ferns (Bower 1923) involves the formation of six partitioning walls which divide the spore mother cell into four equal compartments arranged in a tetrahedrally symmetrical fashion. In a sphere divided in this manner the four lines (edges) which internally limit the six planes partitioning the sphere meet at a point which would be the center of symmetry of a regular tetrahedron inscribed in the sphere. The angle between any two of these lines is the tetrahedral angle or Maraldi's angle (Thompson 1942) of 109° 28′ 16″. The spore mother cell of *Osmunda regalis* divides in this manner (fig. 6). Each spore at its earliest stage is a triangular spherical pyramid with its apex at the center of symmetry of the spore mother cell.

In investigating the behavior of the cytoplasmic inclusions during meiosis in *Osmunda regalis*, it became evident very early that the pattern of behavior was markedly different from that of *Nephrodium* and *Onoclea*. It was also noted that this difference was closely related to the fact that the *Osmunda* spore mother cell divided into a tetrahedral spore quartet rather than the type of quartet characteristic of *Nephrodium* and *Onoclea*. It is the object of this paper to describe the behavior of these inclusions during meiosis in *Osmunda* and to relate their behavior to the tetrahedrally symmetrical arrangement of the newly formed spores.

Materials and methods. Fertile tips of *Osmunda regalis* fronds, bearing sporangia containing desired stages in sporogenesis, were collected from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a wild stand of this species in northern New York City. Segments of these not larger than 5 mm. in length were fixed from twelve to twenty-four hours in a solution freshly prepared as follows (Huseby 1946):

10% commercial formalin	100 ml.
Normal sodium hydroxide	1 ml.
Pyrogallol	7 g.

Pieces were then rinsed briefly in running water, dehydrated with ethyl alcohol, cleared in xylene and embedded in a paraffin medium consisting of 90% 56–58° Fisher Tissuemat, 5% beeswax and 5% bayberry wax. Sections 2–4 micra in thickness were cut without difficulty.

Sections were mordanted 24 hours in 2% ferric alum, rinsed in distilled water and stained 24 hours in 0.5% hematoxylin prepared from a 2% stock solution ripened in direct sunlight for three weeks. Destaining was accomplished in 2% ferric alum, the progress of the differentiation being followed under microscopic observation. Following a 30 minute wash in running tap water, slides were dehydrated in ethyl alcohol, cleared in xylene and mounted in gum damar.

Observations and photomicrographs were made using a Spencer 97X, 1 mm. achromatic oil immersion objective with a Spencer model 320 microscope lamp as the light source. A Bausch and Lomb Euscope with photomicrographic attachment was used for taking the photographs. In the absence of a shutter on this apparatus, the relatively slow film (Kodak Panatomic-X) was exposed by turning the light source on and off. With a yellow filter (saturated solution of picric acid), exposures of 1–3 seconds yielded satisfactory negatives.

Observations. The relatively uniform distribution of granular inclusions throughout the cytoplasm of the spore mother cell is lost by the time

FIGS. 1–6. Diagrams illustrating the structure of the young spore quartets of *Onoclea* and *Osmunda*, and stages in the organization of the undivided and divided spore mother cell of *Onoclea*. FIG. 1. *Onoclea* type quartet. FIG. 2. *Onoclea* type spore mother cell. Interphase following first meiotic division. FIG. 3. *Osmunda* type quartet. FIG. 4. *Onoclea* type quartet drawn to show internal cell wall relations. FIG. 5. *Onoclea* type quartet after completion of cytokinesis, illustrating persistence of mitochondrial plate. FIG. 6. *Osmunda* type quartet drawn to show internal cell wall relations. FIGS. 7–15. Diagrams illustrating changes in the distribution of cytoplasmic inclusions during the second meiotic division in *Osmunda regalis*. FIG. 7. Disc shaped plate characteristic of interphase following first division. FIG. 8. Folding of disc occurring during anaphase II. FIG. 9. Development of the two secondary plates occurring in telophase II. FIG. 10. Completed six-partite figure of interphase following division II. FIG. 11. Interphase following first division. Identical to *Onoclea* type (fig. 2). FIG. 12. Anaphase II. Diagram illustrates a sectional view of the folded plate of figure 8. FIG. 13. Telophase II. Diagram illustrates formation of the two secondary plates shown in figure 9. FIG. 14. Second nuclear division completed. Section is drawn through and parallel to the surface of the vertical plate of figure 10. See also figure 22. FIG. VE. Cytokinesis completed. Section drawn parallel to vertical plate of figure 10, but not through it. See also figure 23.

the metaphase of the first meiotic division is reached. At this stage, the inclusions tend to be localized around the equator of the cell, and in a pole-to-pole section, appear concentrated in the cytoplasm at the edge of the equatorial plate of chromosomes (fig. 16). During the anaphase and telophase of the first division, these laterally concentrated granules invade the spindle area (fig. 17) and at the interphase following the first division form a compact plate lying between the daughter nuclei (fig. 18).

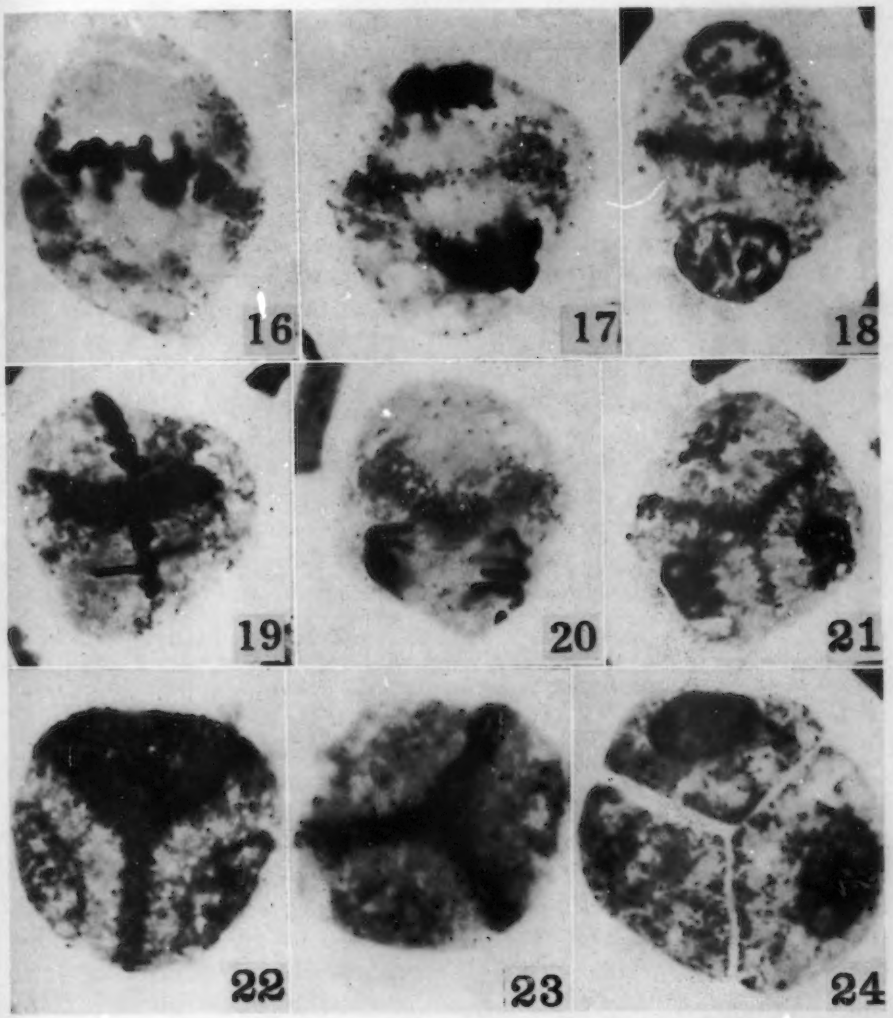
The second meiotic division proceeds simultaneously in both halves of the spore mother cell, with the spindle axes, however, at right angles to each other. At metaphase the mitochondrial plate assumes a thicker and more compact appearance (fig. 19).

The difference in the orientation of the spindles of the second division becomes clearly evident during anaphase, at which stage a section cutting one figure longitudinally will show only the spindle area of the other, with the chromosome groups of this figure being above and below the plane of the section (fig. 20). During anaphase II a further change in the character of the mitochondrial plate is noted, and this appears to anticipate the eventual tetrahedral partitioning of the spore mother cell. In cells selected to show a long section through one of the anaphase II figures, the section through the mitochrondrial plate was invariably V-shaped, with the apex toward the mid-point of the spindle (fig. 20). The three-dimensional interpretation of this consistent feature will be considered in the discussion.

In similarly selected sections of telophase II, a secondary plate of mitochrondria extends from the apex of the primary mass across the spindle area of the second division (figs. 13, 21).

When the nuclei resulting from the second division have reached interphase, the tetrahedral symmetry of the spore quartet is clearly in evidence (figs. 22, 23). The granules originally composing the compact mitochondrial plate of interphase I have become incorporated into six plates which divide the spore mother cell into the tetrahedrally symmetrical spore quartet (figs. 10, 22, 23). Figure 22 shows a particularly favorable section through one of the plates and parallel to its surface, and also through two of the spores and one of the plates limiting them. The mitochondrial plates at this stage appear to be two-layered, presumably indicating that cytokinesis has been accomplished. Later, as the young spores become clearly distinct, mitochondrial localization becomes less marked and is eventually lost (fig. 24).

Discussion. During the first meiotic division in *Osmunda regalis*, the cytoplasmic inclusions follow essentially the same pattern of behavior as in *Onoclea* and *Nephrodium*, with the termination of this division characterized by the presence of a sharply limited plate of granules between the interphase nuclei. The three dimensional interpretation of numerous sections of this stage is that this granular mass is in the form of a disc (fig 7). From



FIGS. 16–24. Photomicrographs of sections of various stages in the division of spore mother cells of *Osmunda regalis*. Fixation in formalin-pyrogallol. Iron hematoxylin stain. Thickness 3 micra. Magnification ×1746. FIG. 16. Metaphase I, showing concentration of inclusions peripheral to the equatorial plate of chromosomes. FIG. 17. Telophase I, showing early stage in the formation of the mitochondrial plate. FIG. 18. Interphase following the first division, showing completed plate of granules. FIG. 19. Metaphase II, showing increase in the thickness of the plate. FIG. 20. Anaphase II, showing folding of the plate. FIG. 21. Telophase II, showing folding of the plate and the formation of a secondary plate in lower half. FIG. 22. Second nuclear division complete. Section taken through and parallel to the surface of one of the six plates present at this stage. FIG. 23. Stage comparable to figure 22, but with the section showing three cells and cross sections through three plates. FIG. 24. Section through an older quartet, showing loss of localization of inclusions, and persistence of tetrahedral symmetry.

this point on, the kinetics of this mass of cytoplasmic inclusions in *Osmunda regalis* is distinctly different from that observed in *Nephrodium* and *Onoclea*. In the latter species, this mass retains its identity until both divisions have been completed, whereas in *Osmunda regalis*, prior to the termination of the second division, it becomes reorganized into a 6-partite figure, which divides the spore mother cell into the four symmetrically arranged portions which are to become the young spores (fig. 10).

On the basis of numerous sections of anaphase, telophase and interphase II, the transformation of the disc into the 6-partite figure is accomplished as follows: Visualizing the granular plate in the horizontal position, the spindle axes of the second division will be parallel to it, but at right angles to each other. During anaphase II, this plate folds in two ways; downward, with the down-turned parts tending to enclose the spindle of the lower figure, and upward, with the up-turned parts tending to enclose the spindle of the upper figure (figs. 8, 12, 20). With the spindles of division II at right angles to each other, this two-way folding of the disc results in the four-planed arrangement shown in figure 8. A section through the long axis of one of the division figures at this stage shows the granular mass as a "V" with its apex toward the center of the spindle shown in long section (figs. 12, 20).

During telophase II, the formation of the two additional plates needed to complete the 6-partite figure is accomplished. Granules appear to migrate from the center of the "V", accumulate across the center of the spindles, and eventually form two new plates in the upper and lower halves of the spore mother cell (figs. 9, 13, 21). Since the granules of these secondary plates first appear across the centers of the spindles, and since the spindles are at right angles to each other, these new plates are likewise at right angles to each other (figs. 9, 10). By the time the nuclei have reached interphase, the granular plates separating them appear to be two-layered, possibly indicating completion of cytokinesis (figs. 14, 22, 23), although the distinctness of the limits of the young spores is not evident until later (figs. 15, 24).

An analysis of the geometry of symmetry was published by Breder (1947). Spherical symmetry is therein defined as that form of solid symmetry which treats of objects with regard to the duplication of parts based upon equal central polyhedral angles. Tetrahedral symmetry, then, is spherical symmetry based upon the central angle of the simplest polyhedron, the regular tetrahedron. The relation of symmetry patterns of pollen grains to the tetrahedral arrangement of the newly formed microspores has been considered by Wodehouse (1929). He states that in dicotyledonous plants, regardless of the orientation of the spindles, the daughter nuclei generally tend to take positions as far from each other as possible, within

the confines of the pollen mother cell wall. This results in the tetrahedral arrangement.

In analyzing the cytological origins of the tetrahedral symmetry of the *Osmunda regalis* quartet and comparing these with the kinetics of sporogenesis in *Onoclea,* the problem of least surfaces enters the picture. The tetrahedral arrangement is the most economical of surfaces separating the four parts of the sphere. The six planes separating the four parts tetrahedrally have less surface than do the three planes dividing the sphere as in *Onoclea.*

A reasonably accurate model of the young spore quartet of *Osmunda regalis* (figs. 3, 6) can be made by stacking four clay spheres tetrahedrally, or cannon-ball fashion, and then manually compressing them into a common sphere. If the pellets have been powdered with talc prior to compression, they will readily separate as four equal triangular spherical pyramids.

The *Onoclea* type arrangement cannot be modeled so readily. If the clay spheres are arranged tetragonally, that is, the four in one plane, and then compressed into a sphere, four equal wedges will be formed, but their long axes will all be parallel. To duplicate the *Onoclea* arrangement, one pair must then be rotated 90°.

In both the *Onoclea* and *Osmunda* type of division, the relative positions of the nuclei formed by the second division are the same, as is the relation between the spindle axes of this division. The problem which here arises is why, when the young spore nuclei of both these species occupy similar positions within the spore mother cell, cytokinesis results in a tetrahedrally symmetrical spore quartet in one instance and not in the other. Although the *Osmunda* type represents the simplest least-surface arrangement, readily duplicated by compressing tetrahedrally stacked clay spheres, it is not believed that external pressures acting upon the dividing spore mother cell are responsible for bringing this about. The basis for this interpretation is the fact that the organization of the cytoplasm into the tetrahedral arrangement precedes cytokinesis, which organization could scarcely be influenced by external pressures. In *Onoclea,* the persistence of the mitochondrial plate until the completion of the second division suggests an inherent cytoplasmic tendency to produce a spore quartet of the other type. In both these species, the organization of the spore mother cell cytoplasm precedes cytokinesis, and this organization foreshadows the type of spore quartet eventually produced.

SUMMARY

1. Fertile tips of *Osmunda regalis* fronds containing meiotic stages in sporogenesis were collected, fixed for mitochondrial preservation, sectioned at 2–3 micra and stained with iron hematoxylin.

2. Study of these sections indicated that through interphase I, the behavior of the cytoplasmic inclusions is essentially like that previously observed in *Nephrodium* and *Onoclea*.

3. Sections of later stages show that in *Osmunda regalis*, the compact plate of inclusions characteristic of interphase I is, by folding and by formation of two additional plates, converted into a 6-partite figure, dividing the spore mother cell into four tetrahedrally arranged spores. The manner in which this is accomplished is described.

4. *Onoclea* and *Osmunda* are compared and discussed. The difference in the kinetics of the cytoplasmic inclusions during sporogenesis in these species, and the relation of this to the type of quartet produced, suggest an inherent tendency present in the cytoplasm of the spore mother cell, which tendency evidences itself prior to the completion of cytokinesis.

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PRESERVATION AND GERMINATION OF THE POLLEN OF GINKGO UNDER STERILE CONDITIONS[1]

WALTER R. TULECKE

Newcomer (1939) has maintained the pollen of *Ginkgo biloba* in a viable condition for a period of one year and has obtained apparently normal ovule and embryo development following pollination with the preserved pollen. Newcomer reported no success in germinating the preserved pollen on any of a variety of culture media, although Kuhlwein (1937) obtained germination of the fresh pollen of *Gingko* in a relatively simple sugar solution. The technique employed by Newcomer involved the desiccation of the pollen over calcium chloride at 7° C.

Other methods for storing pollen have been advanced by Holman and Brubaker (1926), Nebel (1939) and others and, in general, the successful methods have depended to a great extent on the proper drying of the pollen and the use of low temperatures. A portion of the extensive literature on pollen preservation as well as the work of Kuhlwein (1937), Branscheidt (1939), LaRue (1954) and the early workers in the culture of cycad and conifer pollen has been reviewed elsewhere by the author (1953). Outlined here is a method for the sterile preservation and *in vitro* study of the pollen of *Ginkgo*, which, it is hoped, may be applicable to the study of other pollen, such as those which are shed annually or those which have a long maturation period following germination.

Pollen which was to be preserved was obtained by selecting strobili or clusters of sporangia which were plump and yellowed, but unopened. The strobili were surface-sterilized by brief agitation in an alcoholic solution for 10–15 seconds, followed by the prompt removal of the alcohol and the use of a 20% Chlorox (hypochlorite) solution for 20 minutes. The alcohol used was 70–95% ethyl alcohol and it was used as a wetting agent because the heavy cuticular layer of the sporangium was not easily wetted by aqueous solutions. After removal from the Chlorox solution, the strobili were blotted with sterile paper toweling and either cultured directly for fresh pollen studies or put up for preservation and later use. It was important to remove the excess fluid from the strobili because the adhering fluid would only delay desiccation and contribute to the deterioration of the pollen before it was fully preserved. The sterile strobili were transferred to small desiccator bottles (fig. 1). The desiccators were made from one-half ounce Armstrong Capsule Bottles with a screw-cap top. A one-half inch

[1] Part of a thesis submitted to the Graduate School of the University of Michigan in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

layer of calcium chloride was placed in the bottle and a pollen carrier made of aluminum foil was placed on top of the desiccant. Sterilization of the desiccators was accomplished by dry heat in an oven at 200° C. for

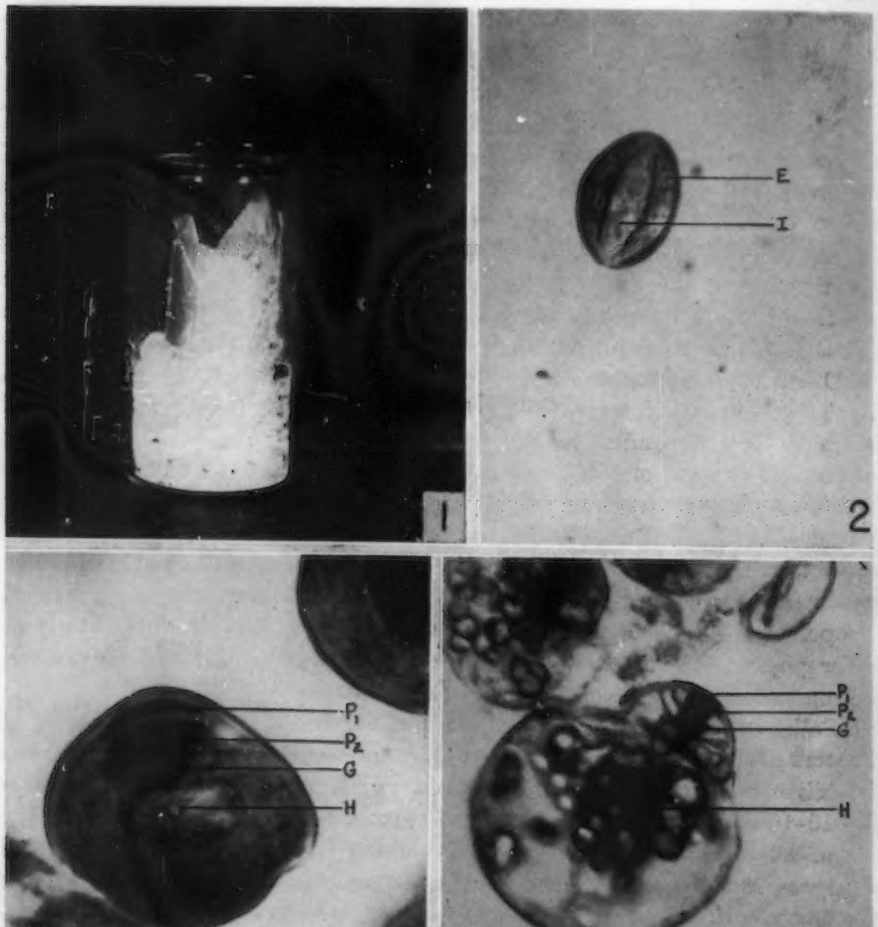

FIG. 1. The dessicator used for pollen storage. approx. nat. size. FIG. 2. Ventral view of the monocolpate pollen grain showing the longitudinal slit in the exine wall (E) and the intine layer (I) which is visible through this slit. 640×. FIG. 3. Mature pollen at shedding stage: evanescent prothallial nucleus P_1, persistent prothallial nucleus P_2, generative cell nucleus G, and the tube nucleus H. 1500×. FIG. 4. An early stage in pollen germination. The tube nucleus (H) is obscured by accumulated starch. 170×.

15 minutes; this effectively drove off any moisture taken up by the calcium chloride during preparation.

One or two sterile strobili were distributed to the pollen carrier of each desiccator and the latter were tightly capped and immediately placed in the refrigerator at 5° C. Dehiscence of the sporangia occurred through the withdrawal of water by the desiccant and the release of the pollen was completed within 12 to 30 hours under these conditions.

Two details may be singled out from this method of pollen storage which distinguish it from Newcomer's original procedure. The first is the technique which permits sterile culture of the pollen and thereby protects the pollen from the attacks of psychrophilic microbes. The second is that anthesis is obtained in the cold. Dehiscence at a low temperature reduces the metabolic rate of the pollen grains to a minimal level while they are still contained within the sporangium, and the method maintains the low rate after the grains are released. Thus, there is little chance for the damaging effects of simultaneous high temperature and partial desiccation to operate to the detriment of pollen viability.

Several media were adequate for the germination of the fresh and the preserved pollen, but one medium was especially well-suited for this purpose. It was a modification of White's basal medium (1943) and it was made up as follows. A stock solution of inorganic salts consisted of magnesium sulfate 14.4 gms., calcium nitrate · $4H_2O$ 10.4 gms., sodium sulfate 8.0 gms., potassium nitrate 3.2 gms., potassium chloride 2.6 gms., and sodium dihydrogen · $4H_2O$ 6.6 gms. in 4 liters of double distilled water. One hundred cc. of this solution were used per liter of medium. The trace elements of White's solution were replaced by Nitsch's (1951) minor elements to which 25 mgm. of cobalt chloride were added. One cc. of this minor element stock solution was used per liter of medium. A Seitz-filtered fraction included yeast extract 2.5 gms./L, indole acetic acid 1 mg/L, glycine 7.50 mg./L, niacin 1.25 mg./L, pyridoxine 0.25/L, and thiamin 0.25 mg./L. Two percent sucrose, .8% agar and 4 cc. of a 2.5 gm./L solution of ferric citrate were added to complete the medium. The pH after autoclaving was about 6.0.

The technique for culturing the preserved pollen was comparatively simple because the pollen was available in powder form after dehiscence had taken place. It could be cultured by dusting on the surface of nutrient agar, by hanging drops, or by suspending in a solution and delivering aliquots as needed. Thus, the powdered pollen afforded considerable ease in handling over the method used for fresh pollen, which was essentially that of LaRue (1954). The fresh pollen was cultured intact in the sporangia after surface sterilization, and dehiscence of the pollen took place as the pollen germinated and broke open the sporangia.

A comparison of the percentage of germination of the fresh and the preserved pollen gave values of approximately 50–60% for the fresh pollen

and 35–45% for the preserved. It is suggested that the discrepancy which is noted may be the result of the preservation process killing the immature grains.

The limitations of this preservation technique as far as other pollen is concerned have not been investigated. However, there is some evidence from experiments performed by Dr. C. D. LaRue at the University of Michigan, using this technique, which suggests that the method will work in the case of the pollen of *Taxus, Ceratozamia, Juniperus,* and *Podocarpus,* but not for *Ephedra* or *Cupressus.*

SUMMARY

1. A method has been outlined for obtaining and preserving the pollen of *Ginkgo* under sterile conditions.

2. The advantage of the technique has been emphasized as the lowering of the metabolic rate of the pollen while it is held within the sporangium, followed by subsequent dehiscence at a low temperature.

3. Germination of both the fresh and the preserved pollen, the latter after storage for more than two years, has been successful on several media, one of which is outlined in detail.

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TORREYA

FIELD TRIP REPORTS

March 27. Queens County, N. Y. for bird life. House finches were "lifers" for most of those present. Hooded mergansers, gadwalls, and green-winged teals represented the waterfowl except for an irate swan that was determined to drive a Canada goose from Valley Stream Lake. Attendance 14. Leader, Harriet J. Brown.

July 4. Many Swamps Trail, Rockland County, N. Y. This trip may be considered as rained out although a stop was made at, what the leader believes is the only station in the county for *Euphorbia Esula*. Attendance; Torrey 4, Rockland-Audubon 1. Leader, J. Harry Lehr.

July 25. Piermont Marshes, Rockland County, N. Y. An exploratory trip into this area in August 1953 produced a number of species at the northernmost limit of their range in New York State. These were: *Spartina cynosuroides, S. patens, S. alterniflora, Cyperus odoratus, Scirpus acutus, Iva frutescens, Pluchea camphorata,* and *Eclipta alba.* No additions to the above list were made on this trip although most of last year's records were substantiated. Attendance; Torrey 6, Rockland Audubon 10. Leader, J. Harry Lehr.

BOOK REVIEWS

Manual of the Plants of Colorado. For the Identification of the Ferns and Flowering Plants of the State. By H. D. Harrington, 676 pp. Sage Books, Denver, 1954. $8.00.

The book makes a poor first impression. From the less than two-page introduction by the author, the rather unfavorable conclusion is reached that the manual was compiled largely from other Floras and monographs without a great deal of field or herbarium study on the part of Dr. Harrington. Comments such as that the "citations [have] not all . . . been actually checked or verified," "use of the terms 'variety' and 'subspecies' follows the particular one used by the author who was followed," and "specific descriptions have been made as complete as possible and have been checked as carefully *as time permitted* . . ." (italics the reviewer's) are responsible for such an impression.

The rest of the 10-page introduction is devoted to an interesting discussion by David F. Costello, of "Vegetational Zones of Colorado" in which it is claimed that "because of the extreme topographic relief in Colorado, vegetation zones are sharply delimited and easily recognized." The state is divided into three main Areas—1. The Plains Area, consisting of Mixed Prairie and Sand Sagebrush; 2. The Semi-desert Area, consisting of Greasewood, Saltbush, and Sagebrush; and 3. The Mountain and Plateau Area, of Pinon-Juniper, Mountain shrub, Ponderosa pine, Spruce-fir, and Alpine. The areas described seem natural enough and rather easily distinguishable. One wonders why Dr. Harrington saw fit to include this ecological discussion since it is completely disregarded by him in treating plant distribution in the state. Instead, Colorado has been divided, for his purpose, into nine equal areas set off on the map by two horizontal and two vertical straight lines, and plant ranges are given in terms of these completely arbitrary segments of the state.

The format of the work is not pleasing. The offset-printed pages, 9 × 11 inches, apparently set a new record for manual size. The print is probably not too fine, but the lines, over 6½ inches in length, are closely crowded and a little hard to follow. It is obvious that much has been sacrificed in the effort to conserve space. One wonders, however, if the space gainable by doubling the columns of the 14-page index might not have been

used more effectively by allowing space between successive species. Such criticisms can not be construed as reflecting on the author.

An unfavorable first impression can easily be forgotten, however, as the keys and text of the work are examined. The book has been very carefully proof read and is remarkably free of typographical errors. Because of the large pages the keys stand out much more clearly than in any other work known to the reviewer; they all seem to be carefully constructed and readily usable. The author has sensibly used consecutive numbers in heading the successive dichotomies and it is indeed a pleasure to mention how easily the key can be used without loss of time in searching for leads. The exceptional genera that usually cause trouble in a key to families are effectively eliminated one by one wherever they intervene in alien company. It is maddening to the average user of a key to families to find that final choice is too often dependent upon the nature of the mature fruit, whereas the material at hand is nearly always in flower. Hundreds of embryo taxonomists will have reason to be grateful that only three times in the course of the 14-page main key does the word "fruit" head the prongs of a dichotomy, and in each of these cases sufficient supplementary characters are given that fruits are unnecessary for judgment.

When it is mentioned that the author has recognized but twelve species of *Lupinus* for Colorado, and that he has seen fit to treat *Viola bellidifolia* Greene as a variety of *Viola adunca* it will be obvious that he has shown a considerable degree of independence in his interpretation of the flora of his state and that he has perhaps been unduly, or at least unusually, honest in acknowledging how much he has depended upon the works of others in writing his manual. A few of the more complete genera have been treated by specialists, it is noted that C. L. Porter prepared the key to *Astragalus* including its many erstwhile segregates, and that E. C. Smith treated the genus *Salix* and C. W. T. Penland, the genus *Penstemon*.

The reviewer would not feel he had done his duty if he could not find some flaw in what he considers to be a very sound piece of work so, hoping that he will not seem picayunish, he will question the advisability of expecting the average user of the manual to look for the Gymnospermae on Page 1 under "AA. Plants producing seeds and with flowers." He also wishes to express just a little disappointment that the inexperienced user of the work will find it necessary, on Page 12, to grope through seven lines of mystery about alternation of generations to reach the only meaningful part of the description of the Pteridophyta, namely—"no true seeds, fruits, or flowers produced;" here is one place where Dr. Harrington might well have departed from convention.

Approximately 3,000 taxa are treated in the book, none of which is new, although 18 new combinations are proposed, all but one of which represent reductions in rank, a fact which it is believed will give a fair idea of the conservative philosophy pervading the Manual. The author is to be congratulated.—C. LEO HITCHOCK, Department of Botany, University of Washington.

The Microtomist's Formulary and Guide. By Peter Gray. xiii + 794 pages, 87 figs. The Blakiston Co., 1954. $10.50.

The Preface points up the pitfalls of textbook publishing, from the author's point of view. The Introduction sets forth the author's philosophy on the subject incidentally to explaining what is to follow. This explanation must be read carefully because in reducing the great amount of material to reasonable volume it has been necessary to introduce devices of efficiency and economy such as decimal indexing for cross reference. The author's "Method of Giving Literature References" will also demand, and deserve, careful study. It is doubtful if any similar book has ever excelled this one in the accuracy, completeness, and specificity with which reagents are treated. The efforts and hours that have gone into checking formulas and references must be staggering. One gets a smile out of finding a slip-up where the author is treating his own work. On page 97 the author cross references to "E 21.1 Gray 1944" but upon turning to E (Chapt. 27,

Embedding Media) 21.1 on page 646 the reference is found as "Gray 1941." This reviewer is convinced that such slips are mighty few.

Part I, "The Art of Making Microscope Slides," 164 pages, deals with standard methods of technique. After years of continuous laboratory experience wherein several "new" procedures and some new reagents, e.g., the embedding wax cited above, have been developed, the author is still enthusiastic about some of the earliest procedures. Sixteen methods are discussed and one or more specific example of each is written out in detail. It is evident that the author has worked more with students in zoology than with those in botany for of the three specific examples of paraffin sectioning not one is of plant material. In an encyclopedic treatise of this sort it also seems peculiar that only derogatory attention is given to recently developed routines which shorten the time from water to paraffin bath and avoid absolute alcohol. Dioxane and *n*-butanol are condemned to the "pathological laboratory, or for sectioning relatively sturdy plant materials" (p. 96) while tertiary butyl alcohol is apparently only adequately treated by ignoring it. Isopropanol is admitted to a tabulation of "physical properties of dehydrating agents" (p. 623). Either under paraffin method or in the chapter dealing with embedding media one might expect to find Bioloid or Tissuemat but such is not the case. The methods themselves in this part are presented very informatively. The reader must adjust to a characteristic phraseology which confused the reviewer at first. Gray puts slides "on one side" to dry where I would put them "aside" to dry.

Part II is devoted to "Methods and Formulas Used in Making Microscope Slides." Perhaps formulas and methods would have been more expressive since it is actually a tabulation of formulas sometimes with and often without a method of use. It is said that there are 3500 of these. They all have citation of original author and publication or as near to it as was possible to determine. Method of preparation is usually given. This part of the book makes it almost indispensable to the teaching laboratory now that it is available. There are twelve chapters, breaking the formulas into groups such as preservatives, fixatives, etc. It requires 497 pages for these. The list of books and periodicals cited (not the references) requires 11 pages. The index occupies 114 pages. This completely expanded index deserves particular commendation. The painstaking work of the author and the understanding of the publisher have combined to make the vast amount of information readily available.

Orchids should go also to Mrs. Gloria Green who did the illustrations. She apparently saw what the author wanted to show the reader. The printing is excellent; we found only one typographic error. A two column format in Part I has permitted use of smaller type with no sacrifice of reading ease. The binding is up to Blakiston's standard of excellence, making the book a beautiful addition to any shelf.—JOHN A. SMALL.

Histopathologic Technic and Practical Histochemistry. By R. D. Lillie. ix + 501 pages. The Blakiston Co., 1954. $7.50.

This book is a revision and expansion of Histopathologic Technic by the same author (1944). It begins with a chapter on microscopy "to bring in certain practical points in the use of the microscope in which I have found it necessary to instruct technicians and physicians in training in pathology." In 19 pages there is an extraordinarily complete and informative presentation of the subject. Phase microscopy is given a fuller and more understandable explanation than I have previously seen.

Chapter 2, "Equipment," is adequate though not as impressive. It does introduce "Mechanical Tissue Changes" (Technicon) which I have not seen elsewhere and a table of Technicon schedules is included, pages 64–65.

The third chapter deals with "Fixation" and this is followed by "Sectioning" where a more comprehensive treatment of the Carbowax method is included than I have seen previously. We are dealing with a book primarily for clinical technicians and physicians which presumably explains the omission of *n*-butyl and tertiary butyl alcohol as dehydrating agents for paraffin embedding. Dioxane and isopropyl alcohol are included, pages 56–57, and methyl salicylate appears in a tabulated schedule, Table 7.

Benzene and gasoline are given high ratings as paraffin solvents following alcohol or acetone dehydration. Isopropanol, *n*-butanol, and Cellosolve are included as solvents for ester wax, page 68. There is no mention of Tissuemat or Bioloid except a puzzling reference to "Will Corporation's Bioloid" under synthetic resins, presumably as a mountant, pages 104 and 106.

The chapter on "Stains and Staining" deals with the nature and use of specific dyes. To insure this specificity in the prevailing confusion of synonymy the Colour Index No. of each is given. Here, as elsewhere, a citation of original reference is included for most reagents.

Chapter 6, "General Staining and Mounting Procedures," finishes off the microscope slide. It includes a particularly good discussion of mountnig media and a tabulation of refractive indices.

The remaining 15 chapters, 331 pages, are largely histochemistry (animal) and special methods. A series of tables in the last chapter, mostly dealing with buffers, may be useful to any technician. There are 30 tables and 2 illustrations (diagrams) in the book. The 45-page index appears to be adequate. Printing, binding, etc., are satisfactory.

If this book is not on the (botanical) laboratory shelf its availability in the library should be assured. Its usefulness to the general technician is obvious.—JOHN A. SMALL.

The Trees and Shrubs of the Southwestern Deserts. By Lyman Benson and Robert A. Darrow. Line drawings by Lucretia B. Hamilton. x + 437 pages + 34 plates. Figs. 1–84. The University of Arizona Press and the University of New Mexico Press. 1954. $8.50.

This book is really a second edition of Manual of Southwestern Trees and Shrubs which was published in 1944 but has been out of print for some years. No explanation is given for the change in title. In the present book, Dr. Benson is responsible for the systematic botany, Dr. Darrow for geographical distribution and economic notes. Five other collaborators have treated a family or genus of their specialty.

The Introduction includes brief discussions of desert climate, vegetation types, and floristic regions together with enough general botany and definitions to facilitate use of the keys. The classification, keys, figures, and general description arranged systematically takes 345 pages with 33 full page, unnumbered plates inserted. The general format is similar to that of the earlier Cacti of Arizona (Bull. Torrey Club **78**: 471). I do not consider the photographs to be as useful diagnostically as those in the Cacti book. This may be due to the different plant group but the photographs themselves are sometimes inferior. Nine plates are in full color, for better or worse. The book includes 117 genera of trees and shrubs while claiming to exclude the bushy type of herb. There must be three times as many species and varieties. The policy here is said to be conservative. Like the Cacti book, range maps are supplied for many of the species. In addition to the description of each species and variety there are other notes where of interest.

In size this book is a shelfmate for the Cacti. Binding and covers are similar but not identical. This book is done on more finished paper. The two books should add much pleasure to a jaunt in our southwestern deserts.—JOHN A. SMALL.

Practical Mycology manual for identification of fungi. By Sigurd Funder. 146 pages; illustrated. Published by Brøggers Boktrykkeris Forlag. Oslo. Steckert-Hafner, N. Y. 1953. $6.50.

The author promises "an introduction to mycology for beginners . . . interested in identifying the most common fungi they meet in the course of their work." It is

stated that "the steady increase of mycological literature . . . makes the subject more and more unapproachable to non-mycologists" (page 3). This is indeed regretable but it is doubtful if this book will allay the confusion. How much it may facilitate the approach will perhaps depend upon the individual. The Preface implies some restriction by naming "bacteriologists, physicians, veterinary surgeons, biochemists, pharmacists, workers in industrial fermentation, the food industry, agriculture, etc." (page 3). It should now be said that if you are expecting to identify fungi generally this book is not for you. It deals largely, if not exclusively, with pathogenic forms. It proposes to employ microscopic characters. This is rather essential in studying the Actinomycetes, Myxomycetes, Phycomycetes, Ascomycetes, Fungi Imperfecti, and the Rusts and Smuts. Only *Polyporus* (?) and *Armillaria mellea* remain (as the fungi are here presented) for macroscopic identification, a rather easy separation.

Pages 13 through 24 are devoted to "Fundamentals of Elementary Mycology." The diagrams and text do a good job of it. Incidentally, the diagram, Fig. 31, is almost certainly *Amanita* though unnamed. Some of the book's intended clientele might well learn that genus. Beyond page 24 we are dealing with identification but except for "A Key to the Human Mycoses by Morris A. Gordon" we are dependent upon a series of diagrams each representing a genus and on a scale such that four can occupy a $7\frac{1}{4} \times 10$ inch page. There are three sections of such diagrams: General Mycology, Medical Mycology, and Plant Pathogenic Fungi. Within each section the arrangement is systematic with the Imperfecti following the Ascomycetes.

Part Three is a Table of Classification occupying a two page spread. Beginning with the Thallophyta, which the author recognizes as a phylum, the classification branches out through ten subordinate taxa to reach the genus, where 122 are tabulated. This is certainly an incomplete coverage of the pathogenic forms and excludes the more numerous saprophytes. There is a 9 page glossary and a 6 page index. The printing is excellent—relatively large type, well spaced, on finished paper. There is no copyright. The cover is striking and leads one to expect more than is delivered.—JOHN A. SMALL.

Laboratory Manual for General Botany. By Ward L. Miller, South Dakota State College. 133 pages, 1 plate.

General Botany Laboratory Manual. By John W. Hall, University of Minnesota. 218 pages, 46 plates, tabulations, work sheets, key, field trip.

Laboratory Exercises for General Botany. By Herbert M. Clarke and Richard I. Evans, University of Wisconsin. 104 pages, 4 plates, 4 tabulations, key.

Laboratory Manual for General Botany. By Robert W. Hoshaw, Sanford S. Tepfer, and Barbara J. Peebles. Illustrations by Ruth S. Hoshaw, University of Arizona. Part I, 74 pages, 3 maps, 9 plates, graphs, tabulations. Part II, 71 pages, 7 plates, tabulation, key, glossary.

All of the above manuals are lithoprinted by Burgess Publishing Company, Minneapolis. No attempt will be made to review them since botanists in the market for a manual will want to see for themselves. We have compared them with our own Laboratory Outline for General Botany (Edwards Bros.) and we conclude that the course of study at each institution, while presenting the same facts, is a rather personal thing. In addition to the contents noted above, the following observations were made: None of these manuals admits its design to accompany a specific textbook. Only one of them (Wisconsin) uses references (to five contemporary textbooks) with the exercises. It will be noted that only one introduces a field trip; all but one use a key. None of them have an exercise on plant propagation, the use of plants in the garden, on the home grounds, within the home, as arrangements, or as exhibits. Only one (Arizona) recognizes vegetation. None of them include ecology. One of them (Minnesota) includes microchemistry.

In matters of classification there is complete divergence. Arizona and Minnesota follow the modern trend though they are not identical. Wisconsin breaks the plant kingdom into 4 Divisions (algae, fungi, bryophytes, tracheophytes) thence to Classes except for subdivisions of tracheophytes. South Dakota avoids the issue by practically omitting systematic relationship in surveying the plant kingdom.—J. A. S.

NOTES

Professor Marion A. Johnson, a recent president of the Torrey Botanical Club, has been appointed Dean of the Graduate School at Rutgers, the State University of New Jersey. His place as Chairman of the Botany Department has been filled by Professor James E. Gunckel.

The Club learned with deep sorrow of the death of George Harrison Shull on September 29 at Princeton, N. J. Dr. Shull had been ill for some time. His absence will be long regretted by the members of the Club at whose meetings he was so faithful in attendance. A biographical sketch of Dr. Shull will appear in an early issue of the BULLETIN.

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INDEX TO AMERICAN BOTANICAL LITERATURE

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LAZELLA SCHWARTEN

WITH THE COLLABORATION OF THE EDITORS OF THE TAXONOMIC INDEX

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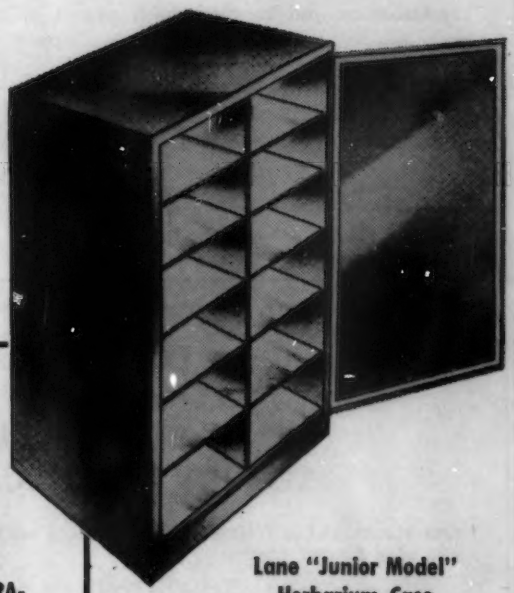

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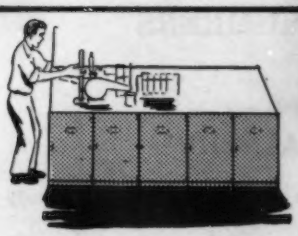

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